AMENDMENTS TO THE SPECIFICATION

Please replace Page 8, lines 13 to page 10, lines 30 with the following:

FIGS. 1A-C illustrate one possible sequence of changes in the structure of a probe during the course of performing the method of the invention. FIG. 1A illustrates one embodiment of the structure of a probe **10** introduced to a cell culture for transport into the cells. The probe generally includes a substrate **20** that is linked to a tag that includes a detection group **21**, such as a fluorescent reporter, a separation modifier M_j **22**, and one or more transport moieties **23**. Once the probe is taken up by the cell, intracellular deacetylases will remove the acetyl groups, as shown in FIG. 1B, resulting in a probe structure **11** that is too hydrophilic to efficiently pass across the cell membrane. FIG. 1C shows a reporter **12** after recognition and action on the deacetylated probe by a reporter gene product, where **24** is any residue S' of the cleaved substrate.

FIGS. 2A and 2B illustrate an embodiment of the methods of the invention for monitoring the effects of a stimulus on transcription from a defined promoter. The figures show a cell **30** which has been transfected with a genetic construct **31** containing a promoter of interest **32** (P) and a reporter gene coding sequence **33** (RCS), which is the coding sequence of a selected enzyme capable of cleaving probes in the probe set of the invention. In this illustration, an external cellular stimulus **34** (S) causes expression of a reporter enzyme **36** (RE), resulting in action on a probe **11** to release a reporter **12**.

FIGS. 3A and 3B depict hypothetical electropherograms of the separation of probe and reporter molecules resulting from the cellular samples of FIGS. 2A and B. FIG. 3A shows a single peak corresponding to the unmodified probe **11**. FIG. 3B shows a second peak arising as a

result of enzyme action on the probe **11** to yield a reporter **12**, catalyzed by expression of the reporter enzyme.

FIG. 4 shows the structures of numerous exemplary reporters having different electrophoretic mobilities.

FIG. 5 is multiple electropherograms showing separation of individual reporters. The figure illustrates obtainable resolution of the reporters, which are identified by their ACLA numbers.

FIG. 6A illustrates an exemplary fluorescent lactam probe for use in the invention. Enzyme products of the probe resulting from successive esterase and lactamase cleavages are shown in FIGS. 6B and 6C. In FIG. 6A, **M** is the separation modifier and **T** is the transport moiety. The cleavage of the transport moieties, **T**, of the probe (FIG. 6A) by an intracellular esterase produces a probe (FIG. 6B), which serves as a substrate for a β-lactamase reporter enzyme. If the reporter enzyme is expressed in the cell, it will cleave the two lactam groups to produce the fluorescent reporter shown in FIG. 6C.

FIG. 7 illustrates another exemplary fluorescent lactam probe for use in the invention where two fluorophores ((D_1, M_1, T) and (D_2, M_2, T)) with overlapping spectra attached to the 7 and 3' positions of a cephalosporin.

FIG. 8A illustrates an exemplary fluorescent lactam probe according to the general structure shown in FIG. 7, in which the uncleaved probe is capable of exhibiting FRET. FIG. 8A illustrates the fluorescent lactam probe where 7-hydroxycoumarin is attached to the 7 position of cephalosporin, and serves as a donor fluorophor to fluorescein, which is attached to the 3' position of the cephalosporin. Enzyme products of the probe resulting from successive esterase and lactamase cleavages are shown in FIGS. 8B and 8C. The cleavage of the probe by β-

lactamase will lead to elimination of the non-fluorescent leaving group, resulting in a change in the mobility of the resulting fluorescent reporter relative to the uncleaved probe.

FIGS. 9A and 9B illustrate an embodiment of the methods of the invention for a multiplexed screen to monitor the effects of a stimulus on transcription from multiple promoters. FIG. 9A illustrates individual construction of separate cell lines **44, 46, and 48** prior to mixing to conduct the screen, where the separate cell lines are transfected with a genetic construct **50, 52, and 54** that includes the promoter P_1, P_2, or P_3 and a reporter coding sequence (RCS_1). FIG. 9B shows the effect of an external cellular stimulus on a mixture of three separate cell lines. Stimulation **70 (S)** of transcription causes expression of a reporter enzyme (RCS_1) in only one of the cell lines having the control of promoter P_1, resulting in cleavage of a probe specifically in the responsive cell line.

FIGS. 10A and 10B depict hypothetical electropherograms of the separation of molecules resulting from the cellular samples of FIGS. 9A and B. FIG. 10A shows three peaks corresponding to the three unmodified probes **56, 58, and 60** contained in the mixture of cells in the untreated state of FIG. 9A. FIG. 10B shows a second peak, corresponding to a single cleaved reporter **64**, arising as a result of modification of the probe by the reporter enzyme in one of the three cell lines of the mixture.

FIGS. 11A and 11B illustrate an embodiment of the methods of the invention for studying protein-protein interactions based on the forward yeast two-hybrid approach. The cells **72** are transfected with a genetic construct **77** having a selected promoter **P** controlling a reporter gene coding sequence **RCS** encoding a reporter enzyme **RE**. The cells are contacted with a probe **78** that is retained within the cell. In this illustration, an external cellular stimulus **S (82)** leads to

disruption of the interaction between two peptides, causing a loss in transcription of the reporter gene.

FIGS. 12A and 12B depict hypothetical electropherograms of the separation of probe and reporter molecules resulting from the cellular samples of FIGS. 11A and B. FIG. 12A shows two peaks corresponding to the unmodified probe **78** and the released reporter **80**. FIG. 12B shows loss of the reporter peak due to disrupted expression of the reporter gene.

FIGS. 13A-C illustrate assembly of transcriptional initiation complexes on copies of the same indicator gene. The complexes have common DNA-binding and RNA polymerase-binding domains, but each has different protein-protein interaction domains. FIG. 13A shows three different first hybrid proteins **85** having first interaction domains **86, 87, and 88** , where proteins **85** bind to the promoter sequence P_1 of a reporter gene **77**. The three different second hybrid proteins having a common transcriptional activation domain **90** having three different second interaction domains **91, 92 and 93** interacts with RNA polymerase II, and are tested for interaction with the first interaction domains **86, 87 and 88**. FIG. 13B shows that in the absence of a binding inhibitor, the specific pairs of hybrid proteins interact with each other to form a functional transcriptional activator that is effective to localize RNA polymerase **95** to the promoter $\mathbf{P_1}$. FIG 13C. shows the induction of transcription of a gene under the control of the promoter $\mathbf{P_1}$.

FIGS. 14A and 14B illustrate an embodiment of the methods of the invention in which an external cellular stimulus (compound **S (82)**) on a mixture of three separate cell lines **100, 101, 102** causes disruption of the expression of a reporter enzyme **RE** in only one of the cell lines, resulting in loss of action on a probe specifically in the responsive cell line. In FIG. 14A, the absence of added agent produces detectable reporters **110, 111 and 112**. In FIG. 14B, the test

compound **S (82)** is added to a mixed cell sample is effective in blocking association of one of the hybrid protein pairs in the cell line **101** resulting in the lack of or reduced levels of the reporter **111**.

FIGS. 15A and 15B depict hypothetical electropherograms of the separation of probe and reporter molecules resulting from the cellular samples of FIGS. 14A and 14B. FIG. 15A shows three peaks corresponding to the three unmodified probes **(105, 106, and 107)** and three peaks corresponding to released reporters **(110, 111, and 112)** contained in the mixture of cells in the untreated state of FIG. 14A. FIG. 15B shows the loss of one of the reporter peaks **(111)** due to disruption of expression of the reporter enzyme in one of the three cell lines of the mixture.

FIGS. 16A and 16B illustrate an embodiment of the invention utilizing a reverse two-hybrid method. In this illustration, an external cellular stimulus **(S (120))** disrupts a protein-protein interaction, resulting in loss of expression of a repressor protein **(tetR)**, and induction of expression of a reporter enzyme **(RE)**. FIG. 16A illustrates an untreated state where the two hybrid proteins interact, leading to the expression of repressor protein **(tetR)** that represses the expression of the reporter enzyme **RE** wherein the probe **78** remains uncleaved. FIG. 16B illustrates the addition of stimulus **120** inhibits interaction between the two hybrid proteins, so that no repressor protein **(tetR)** is produced, allowing transcription of the **RE** gene from a constitutive promoter, and conversion of probe **78** to a reporter **80**.

FIGS. 17A and 17B depict hypothetical electropherograms of the separation of probe and reporter molecules resulting from the cellular samples of FIGS. 16A and B. FIG. 17A shows one peak corresponding to the probe **78**. FIG. 17B shows a second peak arising as a result of action on the probe **78** by the reporter enzyme, to yield a reporter molecule **80**.

Please replace page 23, line 31 to page 24, line 28 with the following:

An exemplary structure of a second class of probes useful in practicing the invention, in which cleavage by β-lactamase causes elimination of a leaving group, is shown in FIG. 7. This structure comprises two fluorophores with overlapping spectra attached to the 7 and 3' positions of a cephalosporin (Zlokarnik). The close proximity of the two fluorophores allows them to exhibit fluorescence resonance energy transfer, or FRET (J. R. Lakowicz, Principles of Fluorescence Spectroscopy, Plenum, N.Y., 1983.). FIG. [7A] 8A illustrates one embodiment of a probe in which 7-hydroxycoumarin is attached to the 7 position of cephalosporin, and serves as a donor fluorophor to fluorescein, which is attached to the 3' position of the cephalosporin. Cleavage of the β-lactam ring of cephalosporin causes spontaneous elimination of the group attached to the 3' position, in this case fluorescein, reestablishing fluorescence emission from the donor by disruption of FRET. The free thiol group remaining on the fluorescein as a leaving group completely quenches its fluorescence. The probe shown in FIG. [7B] 8B contains several transport moieties, which are removed by intracellular esterases to produce the compound shown in [7B] FIG. 8B, and preferably includes a separation modifier, M, attached to a non-quenched fluorescent product of β-lactamase cleavage. Action by a β-lactamase enzyme produces the two products shown in [7C] FIG. 8C. Additional embodiments of this class of probe structures include those reversing the position of attachment to cephalosporin of the donor and acceptor fluorophores.

Probe structures with a leaving group removed by β-lactamase that do not make use of FRET also find use. One embodiment of a probe containing a single detectable moiety is shown in FIG. [8] 7. This probe design comprises a fluorescent detection group D and separation modifier M attached to the 7 position of cephalosporin, and a non-fluorescent leaving group LG

attached to the 3' position of cephalosporin. As with the structure shown in FIG. [7B] 8C, cleavage of the probe by β-lactamase will lead to elimination of the leaving group, resulting in a change in the mobility of the resulting fluorescent reporter relative to the uncleaved probe.